Yeast Probiotic Supplementation in Nursery Pigs in a Challenged Environment

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Lay Abstract: The poor intestinal health, due to many stress factors during weaning, reduces growth and development while in the nursery. With sub-therapeutic antibiotics no longer an option to combat this challenge, probiotics are considered one way to increase gastrointestinal health and promote growth. A 2 x 2 factorial designed experiment looking at dietary intervention with a probiotic and a non-challenge or challenge environment was completed. One hundred and sixty piglets were blocked by weight and sex and randomly assigned to either control diet or supplemented diet in a clean or dirty nursery setting. We hypothesize the piglets with the probiotic in their diet will perform higher in both environments in terms of growth, feed efficiency, and combating illness.

Introduction

At weaning, piglets are exposed to many environmental, social and dietary stressors, which result in decreased feed intake, poor performance and increased risk of disease (Pluske et al., 2018). Intestinal health issues during the weaning period are particularly prevalent because multiple stressors that can culminate in the opportunity for pathogens to out compete healthy commensal bacteria for nutrients and offer a place to colonize within the intestinal tract and cause diarrheal diseases (Pluske et al., 2013). The development of intestinal disease is caused by harmful inflammation of the intestinal barrier, and a functional barrier is essential to the overall health, development, and well-being of the pig (Boudry et al., 2002). The intestinal barrier is constructed of tight junctions that control the types of solutes and macromolecules that migrate through the intestinal epithelial cells (Blikslager et al., 2007). When the intestinal barrier is harmed, and tight junction performance is decreased, overall piglet growth and development is greatly stunted due to the inability of intestinal cells to effectively digest, absorb, and utilize nutrients (Boudry et al., 2002; Moeser et al., 2007).

Poor intestinal function leads not only to slow growth and poor feed efficiency but can manifest in diarrheal diseases that are of great economic burden to the swine industry. The acute inflammation that occurs during exposure to intestinal pathogens can be measured through analysis of inflammatory cytokine levels such as TNF-α, or acute phase proteins, which are highly indicative of heightened immune response (Moeser et al., 2007; USDA 2012). Prior to 2017, swine producers routinely fed growth promoting antibiotics at sub-therapeutic levels to maintain gut health and improve growth and feed efficiency. However, on January 1, 2017 use of antibiotics at sub-therapeutic levels became illegal, and a Veterinary Feed Directive (VFD), for the treatment or documented prevention in the presence of identified disease, is needed to place any medications in the feed. Understanding how dietary interventions, such as probiotics, can be utilized as alternatives to antibiotics to enhance intestinal health in livestock needs more investigation.

Probiotics are defined as live microorganisms that when administered in adequate amounts confer a health benefit on the host (WHO, 2013). One type of probiotic, yeast, has been shown to have the potential to alleviate some postweaning problems (Badia et al., 2012; Pluske et al., 2013; Kiros et al., 2018). Yeast products containing *Saccharomyces cerevisiase* are good sources of enzymes, nutrients and growth factors utilized by commensal microbes to create a homeostatic intestinal microbiome (Pluske et al., 2013; Kiros et al., 2018). Furthermore, the changes to the intestinal environment observed when offering probiotics seem to enhance intestinal health and produce positive production responses in piglets (Pluske et al., 2013).

Problem Identification and Justification

Gastrointestinal health issues rank among the highest causes of neonatal morbidity and mortality across most mammalian species, including domestic livestock. Prior to weaning, gastrointestinal distress in the form of scours accounts for 10.2% of pre-weaning piglet mortality, with 47.8% of herds having *E. coli* disease problems in pre-weaned piglets. (USDA. Part I: Baseline Reference of Swine Health and Management in the United States, 2012). Enterotoxigenic *Escherichia coli* (ETEC) is the most common type of colibacillosis of young animals and is overall the second most prevalent disease in pre-weaned swine (Nagy, 2005).

Objectives and Hypothesis

The objective of this research was to determine the benefits of offering Actisaf HR+, a yeast probiotic, in the diet of the nursery pig on the subsequent nursery pig performance and systemic cytokine levels, from weaning through five weeks post weaning. We hypothesized that piglets fed the yeast probiotic would have a greater growth rate and improved feed conversion ratio than pigs fed the control diet. In addition, pigs were housed in one of two nursery environments; 1) control, a thoroughly cleaned and disinfected room, or 2) challenged environment, a room that has not been cleaned following the previous set of pigs. We hypothesized that the piglets in the challenged environment, when offered the

yeast diet will have improved fecal scores (less diarrhea) and improved growth rate, when compared with piglets fed the control diet. We suggested that greatest impact and benefit would be observed in the first week of the trial when weaning stress is the greatest and intestinal health is most compromised; however, maintaining health in the first week may also improve overall production efficiency through the lifespan of the pig.

Methods

One hundred and sixty pigs (initial BW (bodyweight) = 6.78 ± 0.35 kg) were weaned on average at 22 days of age and allotted to one of four treatments in a 2 x 2 factorial arrangement. Pigs were blocked by BW and sex and assigned to one of two diets and one of two levels of environment (8 pens/treatment and 5 pigs/pen) at the Ohio State University Swine Facility. The treatments were as follows: 1) control diet, clean environment; 2) ActiSaf HR+ diet, clean environment, 3) control diet, dirty environment, and 4) ActiSaf HR+ diet, dirty environment. The trial design is designated in Figure 1. The dirty nursery was not cleaned after the previous nursery group was removed. The clean nursery was cleaned following standard cleaning protocol at the Don Scott Swine Research Center. This animal use protocol was approved by The Institutional Animal Care and Use Committee (IACUC) at Ohio State University.

Pigs were fed nursery diets formulated to meet the NRC requirements for nursery pigs (Table 1; NRC, 2012). Diets were fed in three phases (phase 1 from d 0-7, phase 2 from d 7-21 and phase 3 from d 21-35). Diets supplemented with the probiotic included Actisaf HR+ at 0.1% of the diet (1 x 10^{10} CFU/kg diet) in phases one and two and 0.05% of the diet (5 x 10^{9} CFU/kg diet) in phase three. All diets were pelleted and crumbled at the Ohio State University feed mill located in Wooster, OH. Pigs were housed in thirty-two pens in two separate environmentally controlled nursery rooms (18 pens/room) with raised floors. Each pen was equipped with a self-feeder and two nipple waterers allowing ad libitum access to

feed and water. Pig BW and feed disappearance data were collected at the end of weeks one, two, three, and five.

On days three, seven, fourteen, twenty-one, and thirty-five fecal scores (5/pen) were collected. The severity of diarrhea in each pen was be scored on a scale of one to six: one, dry, hard, well-formed feces; two, soft but formed feces; three, pasty feces green or brown in color; four, viscous feces in light color, episodic; five, fluid feces in light color; six, continuous watery feces. Fecal score was averaged across piglets within a pen for analyses. Piglet health, mortality, and morbidity was monitored and recorded throughout the trial. Antibiotic treatments were administered individually and only upon advisement of the staff veterinarian, and all treatments were recorded.

On days three, seven, fourteen, twenty-one, and thirty-five, blood from two piglets, one male and one female, in each pen was collected via jugular vein puncture. The blood was centrifuged, and the serum was pipetted into 2-mL tubes and stored at -80^{o}C for future analysis. Concentration of TNF-α within the serum concentrations was analyzed via Invitrogen Porcine ELISA kits (Fisher Scientific, Hampton, NH) from female pigs only and on days three, seven, and fourteen only for the present study.

Individual pens served as the experimental unit and four pens within each of the four treatments served as BW and sex block. Growth data, weight and TNF-α measures were analyzed as a 2 x 2 factorial arrangement in a randomized block design with repeated measures in time using the PROC MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The model included fixed effects of diet, environment, and phase of diet, and their interactions. Random effects included pen nested within diet, environment and block. Various covariate structures of error were fitted, and compound symmetry was selected based on the lowest Bayesian Information Criterion (BIC). P-values of less than 0.05 were considered statistically significant for this study.

Results

Growth Performance

No differences in average daily feed intake (ADFI) were observed during phase one, two, three or overall from day 0 to 35 (Table 2). However, differences were observed in average daily gain (ADG) with main effects of environment ($P < 0.01$; Table 2) and phase ($P < 0.001$; Table 2). A trend for a diet by phase interaction ($P = 0.08$; Table 2) was observed. Differences in feed:gain (F:G) ratio were observed with an environment by phase interaction ($P < 0.05$; Table 2). Environment influenced overall, d 0 to 35, ADG and F:G ($P < 0.05$ and $P = 0.05$, respectively; Table 2). Additionally, there was a trend for an overall diet effect in ADG from d 0 to d 35 ($P = 0.09$; Table 2).

Fecal Scores and TNF-α Concentrations

There was an effect of environment by day on fecal scores with increased fecal scores (soft stool) on day three and day seven in the dirty environment compared to fecal scores of pigs in the clean environment ($P < 0.001$; Figure 2). No differences in circulating TNF-α concentrations were observed on days three, seven and fourteen in female pigs across treatments ($P < 0.20$; Figure 3).

Discussion

Although it is recognized that probiotics are viable feed additives for livestock diets (Pluske et al., 2013), our work specifically investigated the use of yeast probiotic, ActiSaf HR+, in weanling pig diets when exposed to clean compared to dirty nursery environments. Numerous nutritional approaches have been investigated to minimize the post-weaning growth depression after piglets are weaned. Probiotics microbes, yeast, have been shown to improve the stability of intestinal microflora and intestinal permeability of newly weaned pigs (Trckova et al., 2014; Che et al., 2017). Our study was not designed to measure clinical outcomes of gut health, but instead, measured growth response, fecal consistency,

and systemic cytokine concentrations. As hypothesized, the dirty environment did provide a challenge compared to the clean environment which was evident in lower ADG and greater F:G ratio from d 0 to d35 when compared to rearing in a clean environment (Table 2). The changes in ADG and F:G ratio for the present trial are likely reflective of the increased fecal scores observed in the dirty environment on day three and day seven of the trial (Figure 2). The increased diarrhea observed in the challenge environment is reflective of issues observed in the swine industry with poor management or poor cleaning protocols that increase the pathogen load on newly weaned pigs in these environments (Bassaganya-Riera et al., 2001). The diet and environment did not have an impact on circulating TNF-α concentrations. However, there was a trend for a day effect for TNF-α concentration, this was the result of an overall increase of circulating concentrations of TNF-α on day seven when compared to days three and fourteen. Since only the female pigs in each pen have been the analyzed to date, the lack of observed difference in TNF-a concentration in the study could be due to small sample size. Of note, other research groups, utilizing dietary by environmental interactions models have shown environmental effects on circulating cytokines in weaning pigs (Bassaganya-Riera et al., 2001).

In conclusion, the environmental challenge significantly reduced ADG and increased F:G ratio as hypothesized. A dietary impact on growth parameters, fecal scores and circulating cytokines concentrations was not observed. The lack of dietary treatment effects may be due to a small number of treatment replicates (eight per treatment), as power calculations indicated eighteen pens per treatment are needed to observe significant differences in feed conversion ration in nursery pig trials. Another replicate of the trial is needed to increase the power of the experiment.

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Figure 1. Nursery design for environmental challenge and dietary treatment assignments. The west nursery was our clean environment and the east nursery was the dirty environment. Pens highlighted in red represent pens of pigs fed the control three phase nursery diets and pens highlighted in grey represent pens of pigs fed the control, three phase nursery diets supplemented with ActiSaf HR+.

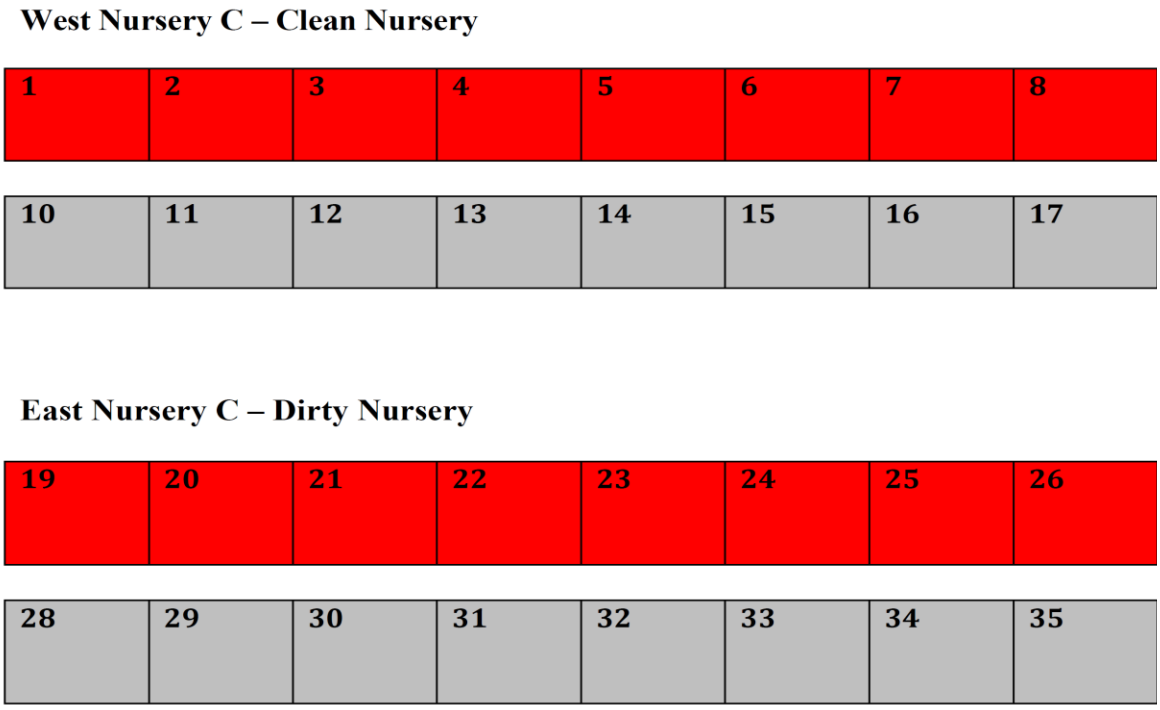

Table 1. Calculated Diet Table

	Phase 1	Phase 2	Phase 3
Weight of Pigs	12 -15 lb	15-25 lb	25-50 lb
Ingredient			
Corn	718	1001	1206
Soybean Meal, Dehull, Sol Extr	380	535	685
Bovine Blood Plasma	80		
Corn DDGS, >6 and <9% Oil	100		
Fish Meal Combined	50		
Milk, Whey Powder	500	200	
Choice White Grease	60	40	40
Calcium phosphate (monocalcium)	15	21	23
Limestone, ground	15.5	22	20
Sodium chloride	6	6	7
L-Lys-HCL	4.5	6	6
DL-Met	2.8	3.2	2.5
L-Thr	1.5	2.2	2.3
Trace mineral premix	3	3	3
Vitamin premix without phytase	5	5	5
Choline chloride 60%	0.7		
HiPhos 2700		0.3	0.3
Zinc oxide	7.8	5	
HP 300 (Hamlet Protein)	50	150	
TOTAL	2000.0	2000.0	2000.0
Required SID Lys:NE Ratio	5.65	5.48	5.04
Calculated SID Lysine Required, %	1.47	1.37	1.25

Table 2. Effect of dietary supplementation of ActiSaf HR+ in a clean compared to a dirty nursery environment on pig performance.

Environment	Clean		Dirty									
Diet	Control	ActiSaf	Control	ActiSaf	SEM	Diet	Environ.	Phase	Diet*Environ	Diet*Phase	Environ* Phase	Diet*Environ*Phase
ADFI, g						0.308	0.265	<0.0001	0.468	0.199	0.6130	0.242
d 0 to 7	228.90	220.29	189.12	206.46	29.3							
d 7 to 21	486.36	533.60	510.74	487.18	29.3							
d 21 to 35	924.29	1009.42	911.51	938.98	29.3							
d 0 to 35	601.57	661.27	603.81	610.42	28.6	0.257	0.403		0.362			
ADG, g						0.120	0.005	<0.0001	0.299	0.078	0.606	0.468
d 0 to 7	60.06	53.90	-8.12	-0.650	23.1							
d 7 to 21	319.07	348.95	313.90	293.34	23.1							
d 21 to 35	547.93	640.34	533.02	570.01	23.1							
d 0 to 35	355.19	406.49	336.47	346.76	17.6	0.092	0.034		0.255			
F:G, g/g						0.690	0.103	0.198	0.795	0.536	0.043	0.907
d 0 to 7	2.84	15.43	-0.71	-8.65	3.8							
d 7 to 21	1.53	1.54	1.69	1.76	3.8							
d 21 to 35	1.72	1.57	1.71	1.66	3.8							
d 0 to 35	1.70	1.62	1.81	1.78	0.07	0.450	0.054		0.665			

Figure 2. Effect of dietary supplementation of ActiSaf HR+ in a clean compared to a dirty nursery environment on fecal scores. The severity of diarrhea in each pen will be scored on a scale of one to five: zero, dry, hard, well-formed feces; one, soft but formed feces; two, pasty feces green or brown in color; three, viscous feces in light color, episodic; four, fluid feces in light color; five, watery feces. This score will be an average of the pen. Bars represent means ± SEM.

Diet P = 0.546
Environment P < 0.0001
Day P < 0.001
Environment*Day P < 0.001

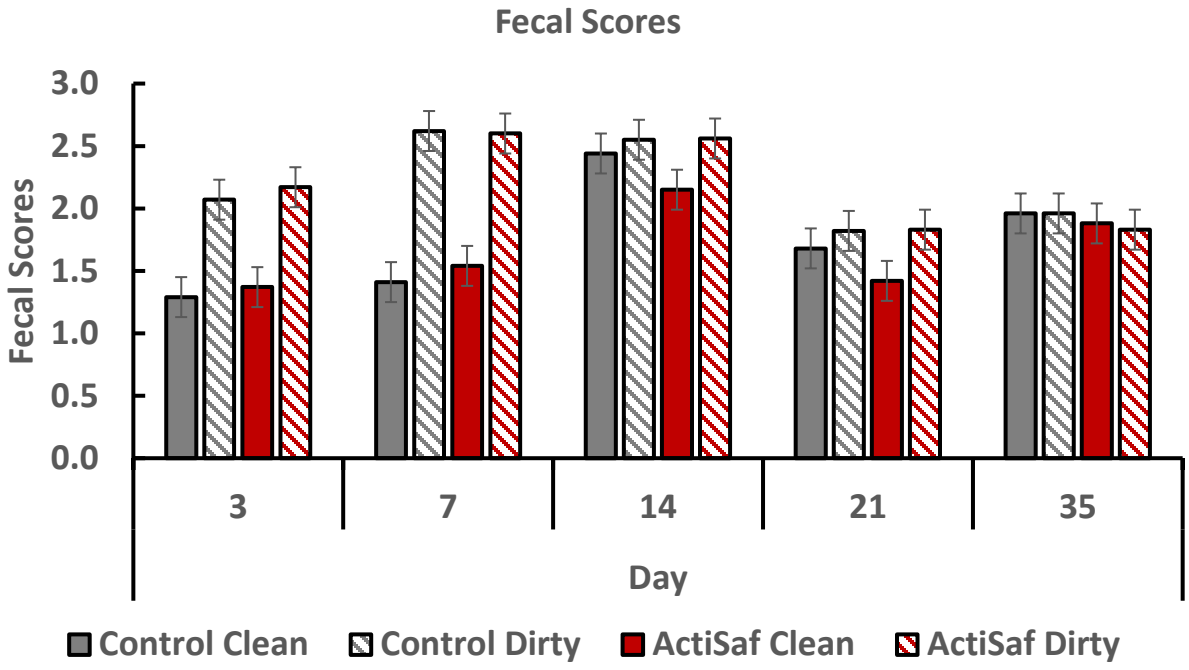

Figure 3. Effect of dietary supplementation of ActiSaf HR+ in a clean compared to a dirty nursery environment on tumor necrosis factor-α. Bars represent means ± SEM.

Diet P = 0.617
Environment P = 0.411
Day P = 0.105
Environment*Diet*Day P < 0.196